

### **REMARKS**

Claim 1 is amended to recite that said proteorhodopsin produces a photocycle when exposed to light of excitation wavelength. Support for the amendment can be found, for example, at page 7, lines 9-15.

New Claims 33 and 34 are supported by page 3, lines 25-26.

No new matter is added in any of the amendments. The Examiner is requested to enter the amendments and re-consider the application.

### **102(a) Rejection**

4. Claims 1, 2, 4, 8, 9, 12-14 and 29 are rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Krebs et al., "Detection of fast light activated H<sup>+</sup> release and M intermediate formation from proteorhodopsin", BMC Physiology, Vol. 2 pp 5-12 (2002). The rejection is traversed in parts and overcome in parts in view of the claim amendment.

(i) Claim 1 and its dependent claims

The Examiner cites SDS gel electrophoresis of solubilized proteorhodopsin in Krebs (pages 7 or 8), and states "where the acrylamide gel (a hydrophilic polymer matrix) is held to immobilize the purified proteorhodopsin and SDS is a detergent solubilizing the pR." The Examiner states that "the claims do not specify the type of information and is considered statement of intended use." In response to the Examiner's comments, Applicants have amended Claim 1 to recite that the immobilized proteorhodopsin is capable of producing a photocycle when exposed to light of excitation wavelength, which distinguishes the SDS gel immobilized proteorhodopsin (PR) in Krebs.

Krebs et al. used SDS/gel electrophoresis to check the purity of PR; the gel was not an optical information carrier. In Krebs et al., the purified PR was analyzed by gel electrophoresis in SDS. PR was denatured in SDS as required by the SDS-PAGE procedures. Although the purified PR was immobilized in the polyacrylamide gel after

electrophoresis, PR was denatured and was not capable of producing a functional photocycle when exposed to light of excitation wavelength.

Other than the denatured PR that was immobilized in the polyacrylamide gel after electrophoresis, Krebs et al. only describe a basic research that examines the physical properties of PR in a solution phase; PR was not fixed to a solid and was not in an immobilized format. Krebs et al. did flash photolysis with reconstituted PR in a solution phase, in which the PR is detergent-solubilized, cellular membrane-free and in a monomer or oligomer form. Krebs et al. extract PR from membrane with a detergent  $\beta$ -octyl-D-glucoside. The column-purified PR was reconstituted into mixed micelles containing 1,2-diheptanoyl-SN-glycero-3-phosphocholine (DHPC), a phospholipid.

At page 6, last paragraph, Krebs et al. state that “The requirement for pR to be in lipid to show fast  $H^+$  release and M formation stems either from a protein/lipid interaction needed to establish a stable, active tertiary structure, or from the need for the phosphate group in DHPC to act as a proton release group.” In the second paragraph of Conclusion at page 7, Krebs et al state “The necessity of reconstituting pR with some lipid before it is capable of photocycling shows that the presence of lipids facilitates pR in assuming its fully active structure.” Krebs et al state that pR needs to be reconstituted with lipids before being capable of photocycling/M state formation, thus Krebs et al. teach away from the present invention of an optical information carrier comprising immobilized PR, which is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form, said proteorhodopsin is capable of producing a photocycle when exposed to light of excitation wavelength. On the contrary, in the present application, Applicants have provided a working example of optical data storage using proteorhodopsin-PVA film, where the PR is detergent-solubilized, cellular membrane-free, and in a monomer or oligomer form. (See application, Example 9).

(ii) Claim 29

Claim 29 is directed to an optical data storage device comprising a light source and an optical data information carrier comprising a solid material having immobilized detergent-solubilized, cellular membrane-free proteorhodopsin, in a monomer or oligomer form, wherein the light source emits a writing light to convert the

proteorhodopsin from a basal state to an M-state. PR immobilized in SDS gel (Krebs et al) cannot produce a functional photocycle, and cannot be converted from a basal state to an M state.

Therefore, Claims 1 and 29 and their dependent claims are not anticipated by Krebs et al.

### **103(a) Rejections**

5. Claims 1, 2, 4, 8, 9, 12-14 and 29 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Krebs et al., in view of Friedrich et al. “Proteorhodopsin is a light driven proton pump with variable vectorality”, J. Mol. Biol., Vol 321(5) pp 821-838 (2002).

As discussed above, Krebs et al do not teach or suggest immobilized PR that is capable to produce a functional photocycle. The combination of Friedrich et al. does not cure the deficiency because Friedrich et al. do not teach immobilized PR that is detergent solubilized, in a monomer or monomer or oligomer form.

The Examiner states that Friedrich et al. teaches the measurement of absorption spectra with PR embedded in 1 mm thick acrylamide gels (page 835, left column). Although Friedrich et al. disclose the purification of proteorhodopsin (PR), **all of the spectroscopic data cited by the Examiner were obtained using PR reconstituted in phospholipid membrane vesicles**, and not using purified PR. This is evidenced by the following passages of the reference. At page 823, 2nd column, where the results from the visible spectroscopy experiments (Figure 2) are described, the reference (at lines 8-10) describes that “yielding a Hill coefficient of 0.67 and a pKa value of 7.68 for reconstituted proteorhodopsin (Figure 2, insert).” This is the experiment where the reconstituted PR was embedded in a polyacrylamide gel. Similarly, at page 835, 1st column, 5<sup>th</sup> paragraph, the reference describes that “Samples for FT-IR spectroscopy with the attenuated total reflection (ATR) technique were prepared by gently reconstituted in PM lipids (3 mg protein per ml solution) on the surface of the internal reflection element (IRE),” which shows that the FTIR experiments were performed with PR reconstituted in a lipid membrane.

Therefore, the combination of Krebs et al., in view of Friedrich et al. does not

produce immobilized PR that is detergent solubilized, in a monomer or oligomer form, and is functional as an optical information carrier. **The detergent-solubilized PR in the form of monomer/oligomer has unexpected advantages over phospholipid vesicle-containing PR in that the former does not cause light scattering, thus providing a good signal-to-noise ratio** (see Application at page 3, lines 25-29).

6. Claims 1-10, 12-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Krebs et al., in view of Friedrich et al., further in view of Hampp et al. '279 and/or Wu et al. "Bacteriorhodopsin encapsulated in transparent solgel glass: A new biomaterial", Chem. Mater. Vol. 5 pp. 115-120 (1993).

As discussed above, the combination of Krebs et al., in view of Friedrich et al. does not produce immobilized PR that is detergent solubilized, in a monomer or oligomer form, and is functional as an optical information carrier (Claims 1, 29 and their dependent claims).

Claims 26 and 28 are directed to a method of optically storing information on a material or a method of producing a three-dimensional optical image for information storage, comprising directing onto only a selected portion of a material containing immobilized proteorhodopsin light of a first spectral range representing optical information to be stored; and exposing the selected portion of the material containing immobilized proteorhodopsin to switch the proteorhodopsin from its basal state to its M state. As the Examiner previously suggested, Claims 26 and 28 have preclude the selected portion being the entire layer. Krebs et al. and Friedrich et al. do not teach or suggest directing light onto only a selected portion.

The addition of Hampp et al., which only disclose bacteriorhodopsin (BR), does not cure the deficiency of Krebs et al. or Friedrich et al. Hampp et al. use native purple membrane patches, which are micrometer sized patches containing a 2D crystal of lipids and BR proteins. Hampp et al. do not teach or suggest PR, let alone detergent-solubilized PR in the form of monomer/oligomer.

Wu et al. only disclose bacteriorhodopsin encapsulated in sol-gel. Wu et al. do not mention proteorhodopsin. Therefore, the addition of Wu et al does not cure the deficiency of other cited references.

7. Claim 11 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hampp et al. WO 00/59731 (US 6,616,964 is English equivalent and used in translation), in view of Friedrich et al. and Beja et al. "Bacterial Rhodopsin: Evidence for a new type of phototrophy in the sea" Science Vol. 289 pp 1902-1906 (09/2000).

To further the allowance of this application, Applicants have cancelled Claim 11.

8. Claims 1, 2, 4, 8, 9, 11, 12-14, 29 and 32 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Krebs et al., in view of Friedrich et al., further in view of Hampp et al. WO 00/59731 and Beja et al.

Claims 11 and 32 are cancelled.

As discussed above, the combination of Krebs et al. and Friedrich et al. does not produced Claims 1, 29 and their dependent claims.

Hampp et al. do not teach or suggest PR, let alone detergent-solubilized PR in the form of monomer/oligomer.

Beja et al. describe basic research that examines the physical properties of PR. Beja et al. do not disclose immobilize PR in the form of monomer/oligomer.

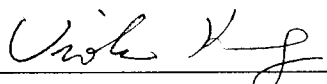
Therefore, Claims 1, 29 and their dependent claims are not obvious over the cited references.

### CONCLUSION

Applicant believes that the application is in good and proper condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 798-3570.

Respectfully submitted,

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